Proceedings of Symposium on Ultrasonic Electronics, Vol. 36 (2015)
5-7 November, 2015

Effect of ultrasonic frequency on the inactivation of yeast
酵母菌の非活性化における超音波周波数の影響

Yoshinori Ike†, Takashi Ikeno, Shohei Ota, and Ken Yamamoto
(Fac. Eng. Sci., Kansai Univ.)
池祥宣†, 池野孝, 大田将平, 山本健 (関西大学 システム理工)

1. Introduction

Bubbles form repeatedly and vibrate in liquid during ultrasonic irradiation in a process known as ultrasonic cavitation.[1-2] Cavitation bubbles are caused by the physical effect of expansion and contraction, through shock waves, micro-jets, shear fields, and density changes, and by chemical effects arising from localized high-temperature and high-pressure fields forming OH radicals and H_2O_2. These effects are inactivated by many types microorganisms;[3-6] however, the mechanisms have not been identified. In this work, we investigated the inactivation mechanism by examining the effects of six ultrasound frequencies on two species of yeast.

2. Materials and methods

The yeasts *Saccharomyces cerevisiae* NBRC 1346 and NBRC 2043 were grown in yeast mold medium (glucose, 10 g; peptone, 5 g; yeast extract, 3 g; malt extract, 3 g; distilled water, 1 L) for a total of 21 h at 30 °C. In all experiments, prior to sonication the concentration of yeast cells was adjusted to about 10^6 cells/mL with a UV spectrometer (UV-1800, Shimadzu). Figure 1 shows the experimental sonication apparatus. A 20 kHz ultrasonicator was used (VC 750, Sonics & Materials Inc.). A disk-type PZT ceramic transducer in a stainless steel cylinder was used at frequencies of 400 kHz, and 1.0, 2.3, 3.4, and 4.4 MHz. The system was operated for up to 15 min with a cooling system to maintain the temperature at 19-21 °C. Experiments were performed at an acoustic power of 10 W in duplicate. Samples were taken after 0, 3, 6, 9, 12, and 15 min of sonication and the yeast cells were examined by colony counting, cell counting, and turbidimetry.

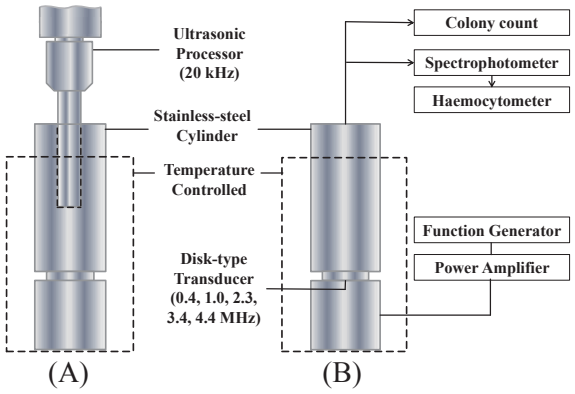


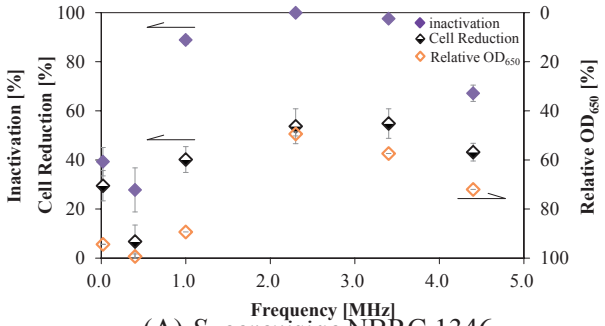
Fig. 1 Schematic of the reactors for ultrasonic irradiation at 20 kHz (A) and 0.4, 1.0, 2.3, 3.4, and 4.4 MHz (B).

k793712@kansai-u.ac.jp

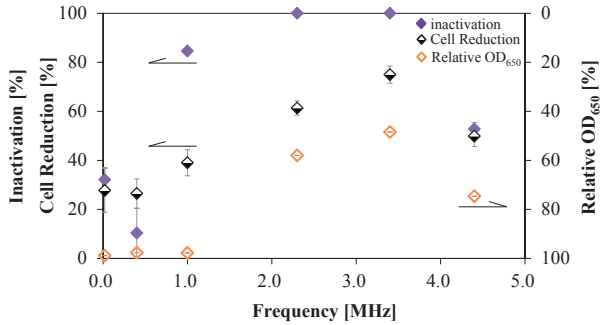
3. Results and discussion

Figure 2 shows the colony counting, cell counting, and turbidimetry of yeast suspensions after 9 min sonication. The highest inactivation was achieved at 2.3-3.4 MHz for both yeast species. Figure 3 shows the formation of chemical species as a function of frequency.[7] The amount of chemical species reached a maximum at 400 kHz and a minimum at 2.3-3.4 MHz. This was because the *S. cerevisiae* cells were destroyed by the physical effect of the ultrasonic cavitation. The oscillation of cells during ultrasonication was studied by Zinin and co-workers.[8,9] They obtained the natural frequency of $f_K \approx 1/2\pi\sqrt{K_A/(\rho a^3)}$ (K_A: the surface area modulus; ρ: the density; a: the radius of the cell). *S. cerevisiae* NBRC 1346 and NBRC 2043 have mean radii of 2.3 and 1.7 μm and elastic moduli of 105 and 144 MPa, respectively. These were measured by scanning probe microscopy (SPM-9700, Shimadzu). For most plant cells, the thickness is approximately 1% of the size of the cell, and Poisson's ratio and density were assumed to be the same as for water. Table I shows the natural frequency of NBRC 1346 and NBRC 2043 calculated with the equation. The natural frequency of *S. cerevisiae* was on the order of several megahertz, consistent with the experimental results. Table II shows the natural frequency of *S. cerevisiae* calculated by the finite element method,[10] and the results showed a similar trend to those obtained by the equation. Therefore, *S.*

cerevisiae was inactivated by the mechanical resonance of the cell wall caused by the bubbles.



(A) *S. cerevisiae* NBRC 1346



(B) *S. cerevisiae* NBRC 2043

Fig. 2 Inactivation and cell reduction of *S. cerevisiae* (105 mL) as a function of frequency during ultrasonication (9 min, 10 W).

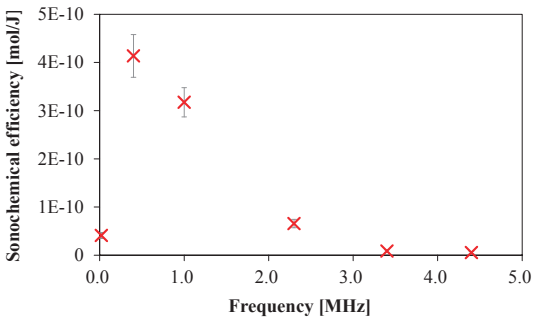


Fig. 3 Frequency dependence of sonochemical effect in 0.1 M KI solution.

Table I Natural frequency of 1% and 5% for the cells calculated with the natural frequency equation.

Cell type	Natural frequency of 1%	Natural frequency of 5%
NBRC 1346	2.3 MHz	5.1 MHz
NBRC 2043	3.7 MHz	8.2 MHz

Table II Natural frequency of 1% and 5% for the cells calculated by the finite element method.

Cell type	Natural frequency of 1%	Natural frequency of 5%
NBRC 1346	0.9 MHz	0.9 MHz
NBRC 2043	1.2 MHz	1.3 MHz

References

1. T. Taeda, I. Oyane, K. Okitsu, M Furuta, Toxicity Evalution of Sonochemical Decomposition Products of Chloroacctone to Yeast Cells by Calorimetric Analysis, Bunseki Kagaku, 55 (9), 701-706, 2006.
2. Hua and J. E. Thompson, Inactivation of Escherichia Coli by Sonication at Discrete Ultrasonic Frequencies, Water Research, 34 (15), 3888-3893, 2000.
3. H. Hao, M. Wu, Y. Chen, J. Tang, Q. Wu, Cavitation mechanism in cyanobacterial growth inhibition by ultrasonic irradiation, Colloids and Surfaces B: Biointerfaces, 33 (3-4), 151–156, 2004.
4. S. Radel, A. J. McLoughlin, L. Gherardini, O. Doblhoff-Dier, E. Benes, Viability of yeast cells in well controlled propagating and standing ultrasonic plane waves, Ultrasonics, 38 (1-8), 633–637, 2000.
5. S. Koda, M. Miyamoto, M. Toma, T. Matuoka, M. Maebayashi, Inactivation of *Escherichia coli* and *Streptococcus mutans* by ultrasound at 500 kHz, Ultrasonics Sonochemistry, 16 (5), 655-659, 2009.
6. K. Yamamoto, P. M. King, X. Wu, T. J. Mason, E. M. Joyce, Effect of ultrasonic frequency and power on the disruption of algal cells, Ultrasonics Sonochemistry, 24, 165-171, 2014.
7. S. Koda, T. Kimura, T. Kondo, H. Mitome, A standard method to alibrate sonochemical efficiency of an individual reaction system, Ultrasonics Sonochemistry, 10 (3), 149-156, 2003.
8. P. V. Zinin, J. S. Allen III, Deformation of biological cells in the acoustic field of an oscillating bubble, Physical Review E, 79, 021910, 2009.
9. P. V. Zinin, J. S. Allen III, and V. M. Levin, Mechanical resonances of bacteria cells, Physical Review E, 72, 061907, 2005.
10. M. M. Zarandi, A. Bonakdar and I. Stiharu, Investigations on Natural Frequencies of Individual Spherical and Ellipsoidal Bakery Yeast Cells, Proceedings of the COMSOL Conference 2010.